Merck & Co., Inc.
Two Bethesda Metro Center
Merck Research Laboratories Suite 700
Bethesda MD 20814
Tel 301 941 1400
Fax 301 941 1406

August 5, 2005

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

RE: [Docket No. 2005D-0203] – Draft Guidance for Industry on Safety Testing of Drug Metabolites; Availability

Merck & Co., Inc. is a leading worldwide human health products company. Through a combination of the best science and state-of-the-art medicine, Merck's Research and Development (R&D) pipeline has produced many important pharmaceutical products available today. These products have saved the lives of or improved the quality of life for millions of people globally.

Merck Research Laboratories (MRL), Merck's research division, is one of the leading biomedical research organizations. MRL tests many compounds as potential drug candidates through comprehensive, state-of-the-art R & D programs. Merck supports regulatory oversight of product development that is based on sound scientific principles and good medical judgment.

In the course of bringing Merck drug product candidates through developmental testing and clinical trials, Merck scientists address issues affected by this proposed Guidance. We have extensive experience in the clinical development of drug candidates and have utilized that experience to author the comments below.

General Comments

Although we commend the Food and Drug Administration (the Agency or FDA) for their commitment of providing guidance to industry concerning nonclinical safety testing, we have major comments concerning this effort. The draft Guidance on safety testing of drug metabolites makes recommendations on when and how to identify, characterize, and evaluate the safety of "*unique*" human metabolites and "*major*" metabolites of candidate drug molecules. Particular concerns are expressed in the document that certain metabolites may not be adequately assessed during the course of standard nonclinical studies because they may occur only in humans ("*unique*" metabolites), or that they may occur at higher levels in humans ("*major*" metabolites) than in the animal species used during nonclinical toxicology testing. While the draft Guidance acknowledges that unique

human metabolites are rare, it fails to recognize that quantitative differences in metabolite formation between species are the rule, rather than the exception. This, in turn, leads to the inappropriate conclusion that additional testing of synthetic metabolites is necessary in many cases.

The peer-reviewed literature[1][2][3] and international consensus guidance documents (e.g. ICH Guidance documents S3A, M3, and S1C[4]) address the issues raised in the draft Guidance. The FDA has participated in the development of and has used these international guidelines effectively in the past and the need for an additional guidance at this time is not clear. However, the wide dissemination of the draft Guidance implies that it represents current FDA thinking on this issue, and reviewers have begun to use the draft Guidance as a foundation for requests relative to new drug development. As noted below, the draft Guidance incorporates ambiguous language that already has led to variable interpretations by Agency reviewers, and the resulting problematic recommendations are certain to negatively impact new drug development.

The most significant problem with the draft Guidance is that it represents a significant departure from the FDA's Critical Path Initiative (http://www.fda.gov/oc/initiatives/criticalpath/). The Agency and the pharmaceutical industry are moving rapidly, in a concerted fashion, to incorporate new scientific and technical tools (toxicogenomics, biomarkers, computer modeling techniques, clinical trial endpoints, etc.) to make the drug development process more efficient. There is no question that implementation of the recommendations contained in this draft Guidance would have far-reaching implications in terms of increased drug development costs and protracted timelines without adding significant value to the benefit risk equation, in direct opposition to the spirit of the Critical Path Initiative.

Our four major concerns with the draft Guidance document are summarized as follows:

1. As noted in the Critical Path white paper, currently there is only an 8% chance of a drug candidate in Phase I clinical trials ultimately reaching the market. The draft Guidance would effectively require that resource-intensive human ADME (adsorption, distribution, metabolism and excretion) studies be conducted at approximately the same time as the Phase I clinical trials in order to identify, synthesize and test any major

---

[1] T. A. Baillie, M. N. Cayen, H. Fouda, R. J. Gerson, J. D. Green, S. J. Grossman, L. J. Klunk, B. LeBlanc, D. C. Perkins and L. A. Shipley, Drug metabolites in safety testing. Toxicol. Appl. Pharmacol., **182**, 188-196 (2002).

[2] K. L. Hastings, J. El-Hage, A. Jacobs, J. Leighton, D. Morse and R. E. Osterberg, Letter to the Editor. Toxicol. Appl. Pharmacol., **190**, 91-92 (2003).

[3] T. A. Baillie, M. N. Cayen, H. Fouda, R. J. Gerson, J. D. Green, S. J. Grossman, L. J. Klunk, B. LeBlanc, D. C. Perkins and L. A. Shipley, Reply. Toxicol. Appl. Pharmacol., **190**, 93-94 (2003).

[4] S3A Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies. M3 Timing of Pre-Clinical Studies in Relation to Clinical Trials. S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals.

metabolites for which "*adequate*" exposure was not achieved in nonclinical animal species. Resource intensive human ADME studies would now be taking place even prior to confirmation of efficacy in humans (Phase IIA). Although the draft Guidance indicates that studies with animal and human hepatocytes may be used for interspecies metabolism comparisons, such data provide only a qualitative comparison of metabolite profiles, and would be insufficient to meet the quantitative *in vivo* criteria from human ADME studies that would serve as the definitive basis for metabolite synthesis and testing. This excessive 'front-loading' of resources is clearly at odds with the recommendations of the Critical Path Initiative.

2. III.A. *Safety Testing and Nonclinical Study Design, Goals of Safety Testing.* Section III.A of the draft Guidance raises the possibility that virtually unlimited resources would have to be committed to the majority of drug development programs. Thus, it is stated that, "*...when a potentially clinically relevant toxicity is observed during standard nonclinical studies, it is prudent to determine if metabolites contribute to that finding. In such cases, we recommend that the metabolites be synthesized and directly administered to the appropriate animal species for further pharmacological/toxicological evaluation.*" Generally, all adverse effects observed in nonclinical studies are assumed to have potential clinical relevance. In addition, a fundamental principle used for dose selection in nonclinical testing is that the top dose should be based on dose-limiting toxicity. This consideration, combined with the above recommendation, will result in the need to test multiple metabolites which, in turn, will necessitate the conduct of multiple CMC programs without any clear benefit for human safety. Additionally, the proposed increase in toxicity testing is counter to current efforts to decrease unnecessary animal usage in research.

To the extent that the toxicity of a drug candidate is mediated by a metabolite, it is our view that the toxicity will be defined adequately by testing the parent compound and demonstrating exposure to the metabolite.

The determination of whether an adverse effect is caused by the parent compound or a metabolite could take years of research and would significantly delay all nonclinical testing programs demonstrating dose-limiting toxicity. To prevent a major increase in drug development costs and a significant increase in the amount of time required to develop new drugs, this text and 'recommendation' should be removed.

3. Section II. *Background.* The basis for the selection of the arbitrary 10% level for consideration for safety assessment may be reasonable for relatively high dose drugs (>100mg) that form reactive intermediates. However, the draft Guidance applies this standard uniformly to all metabolites regardless of pharmacological activity, protein binding, chemical reactivity, structural alerts or other toxicological concerns. There is very little evidence in the published literature for safety problems associated with low

levels of circulating or excreted non-reactive metabolites[5]. Therefore, the application of a low threshold for all metabolites, regardless of their chemical nature, is not scientifically justified based on the arguments presented.

In addition to the above considerations, it should be recognized that many metabolites will prove to be extremely difficult to synthesize in the amounts and purities required for toxicity testing (e.g. regiospecific ring-hydroxylated species). Moreover, many metabolites, including by definition reactive metabolites, may be unstable chemically, thereby precluding toxicity testing of any duration. These synthetic issues may render it impossible to assess the safety of some metabolites, and therefore a simple "10% threshold" for testing all metabolites is impractical.

Industrial sponsors and the FDA alternatively should consider developing a threshold based on the body burden (dose) of the parent drug, since the absolute exposure (metabolite abundance) achieved using the 10% criteria will vary significantly depending on the size of the administered dose. A thoughtful commentary on this approach has been proposed recently by the Pfizer group[6].

4. Section III. Safety Testing and Nonclinical Study Design. Aside from the resource considerations noted above, the proposed toxicology testing of synthetic metabolites raises a number of fundamental concerns from a scientific standpoint. There are many documented examples in the literature where the disposition of a preformed metabolite given to animals or humans differs from that of the corresponding metabolite generated endogenously from the parent, even when the route of administration is the same. Hence, the results of toxicity testing employing such a study design may be misleading, and fail to characterize the true toxicological contribution of the metabolite when formed from the parent. Such complications are evident in three situations, namely (a) the metabolite undergoes sequential metabolism to a downstream product that is toxic, (b) a diffusional barrier to the metabolite exists (e.g. tissue uptake is mediated by an influx transporter), and (c) the locus of formation of the metabolite differs from that at which it causes toxicity.

As an example of situation (a), hepatic exposure to acetaminophen (and the hepatotoxic intermediate to which it gives rise) has been shown to be significantly greater in animals dosed with phenacetin (a metabolic precursor of acetaminophen) than in animals given an equimolar amount of acetaminophen itself[7]. Under situation (b), the importance of diffusional barriers in drug disposition is best illustrated by prodrugs, such as enalapril

---

[5] J. L.Walgren, M. D. Mitchell and D. C. Thompson, Role of metabolism in drug-induced idiosyncratic hepatotoxicity. Crit. Rev. Toxicol., **35**, 325-361 (2005).

[6] D. A. Smith and R. S. Obach, Seeing through the MIST: Abundance versus percentage. Drug Metab. Dispos. – in press (2005).

[7] K. S. Pang and J. R. Gillette, Kinetics of metabolite formation and elimination in the perfused rat liver preparation: Differences between elimination of preformed acetaminophen and acetaminophen formed from phenacetin. J. Pharmacol. Exp. Ther., **207**, 178-194 (1978).

which is hydrolyzed completely in vivo to the active ACE inhibitor enalaprilat. Studies have demonstrated that the hepatic exposure to enalaprilat can be as much as three-fold higher when dosed as enalapril than when given as the preformed metabolite, apparently due to the effects of a dicarboxylic acid transporter[8]. An example of situation (c) is found with certain carcinogenic aromatic amines, which are subject to hepatic N-hydroxylation and subsequent 'transport' from the liver in the form of labile N-glucuronide conjugates to the urinary bladder where the glucuronide is cleaved and local toxicity ensues[9]. The tissue distribution (and toxic effects) of the metabolite dosed as the preformed hydroxylamine would be anticipated to be quite distinct from those of the corresponding metabolite generated endogenously. It should be stressed that the route of administration also can have a significant effect on the toxicity profile of a compound, as has been demonstrated repeatedly by a compendium of toxic effects which differ for agents that have been dosed orally relative to when the same agent is administered by the IV route. Hence, the results of toxicity testing with a preformed metabolite, regardless of route of administration, need to be interpreted with great caution. Unfortunately, this basic scientific limitation is ignored in the draft Guidance.

In addition to the major comments provided above, we are providing the more specific comments below.

Confusion between '*systemic exposure*' and '*dose*'
The definition of '*Dose*' used for decision-making, as outlined in Appendix A: Decision Tree Flow Diagram, is not clear. In addition, the definitions used in the document for decision-making relative to metabolite safety testing vary (*cf* Sections I, II, III.B. and Glossary). Much of the ambiguity arises from mixing two fundamentally different concepts. ICH S.3A clearly indicates that the appropriate metrics for quantification of systemic exposure are plasma concentrations or AUCs (area under the curve) of the parent compound and/or metabolites (Sections 3.2 and 3.8). Footnote 9 of Section 3.8 in ICH S.3A, *Determination of Metabolites*, indicates that measurement of human metabolite concentrations in plasma of non-clinical toxicity studies is important to demonstrate adequate testing of metabolites.

However, the draft Guidance confuses systemic exposure (as defined by ICH S3A) with the excretion of noncirculating metabolites in bile, feces, or urine and treats them equivalently (Section III.B. *Identification of Metabolites*, 2nd paragraph). Subsequently in the same paragraph, it indicates that if conjugated metabolites are detected in excreta, it can be assumed that systemic exposure has occurred. The text concludes that systemic

---

[8] K. S. Pang, W. F. Cherry, J. A. Terrell and E. H. Ulm, Disposition of enalapril and its diacid metabolite, enalaprilat, in a perfused rat liver preparation. Presence of a diffusional barrier into hepatocytes. Drug Metab. Dispos., **12**, 309-313 (1984).

[9] F. F. Kadlubar, L. E. Unruh, T, J. Flammang, D. Sparks, R. K. Mitchum and G. J. Mulder, Alterations in urinary levels of the carcinogen, N-hydroxy-2-naphthylamine, and its N-glucuronide in the rat by control of urinary pH, inhibition of metabolic sulfation, and changes in biliary excretion. Chem.-Biol. Interact., **33**, 129-147 (1981).

exposure to metabolites in plasma, or their presence in excreta, are equivalent criteria for the purpose of determining adequacy of human exposure in nonclinical species. This interpretation differs significantly from international consensus guidelines. Excretory metabolites may represent a measure of exposure for the excretory organ but they may or may not represent a measure of systemic exposure (Line 178 of the draft Guidance acknowledges that they are noncirculating). However, we agree that the measurement of excretory metabolites has value for demonstrating that a metabolic pathway is operative in nonclinical species, and this factor should be taken into account in considering the need for further testing.

The four examples of drug metabolites used to support the use of 10% threshold of drug related material in plasma are not appropriate

Section II, *Background.* All four examples cited in Section II of the draft Guidance involve the formation of chemically reactive metabolites that bind covalently to macromolecules (protein or DNA) and form stable conjugates that are excreted. In the case of the prodrug cyclophosphamide, metabolism leads to phosphoramide mustard which alkylates DNA and thus provides the basis for the cytotoxic effects of this chemotherapeutic agent. In the case of the other drugs mentioned (halothane, felbamate and acetaminophen), years of research were required to identify the reactive metabolites responsible for their toxic effects. More importantly, none of the reactive metabolites of these three drugs are detectable, at any level, in the plasma of nonclinical species or humans, and thus the synthesis and toxicity testing of these reactive species would not be practical. More appropriate examples that involve significant systemic human plasma exposure to circulating toxic metabolites without adequate coverage of plasma exposure in nonclinical species should be provided, if indeed they are available, to justify the recommendations of this draft Guidance. Parenthetically, it should be noted that the toxicity of most (if not all) of the drugs cited above would have been characterized adequately by classical nonclinical safety assessment paradigms.

Definition of a threshold of concern for metabolites

Although the use of systemic exposure (defined as AUC) is the international standard for defining safety margins for drugs, the definition of a threshold of concern for metabolites based upon the percentage of drug-related material in plasma has value. This is the most direct means of evaluating human ADME data from studies with a radiolabeled drug. For technical reasons associated with the quantitation of metabolites in radiometric HPLC profiles and the limitation of the amount of radioactivity that can be dosed to humans (maximum of approximately 100 μCi of radiolabeled drug), it is difficult to reliably quantitate minor metabolites that comprise 10% or less of total drug-related AUC. A threshold of concern for drug metabolites that represents 25% of the drug-related material in human plasma is reasonable based upon reliable quantitation and the likelihood that a metabolite at such levels could represent a substantive toxicological risk. After identification of human metabolites that are above the threshold of concern, an evaluation of the systemic exposure (based upon AUC) should be conducted to determine whether adequate exposure has been established in the test species. Given the uncertainty in

evaluating margins and in the response between species and the long experience where human drugs have been safely developed where the human exposure to the parent drug exceeds the exposure in the nonclinical species, we suggest that an animal/human exposure margin in the range of ≥ 0.25 should be sufficient. However, it should be noted that no consensus has been reached among experts on the magnitude of what constitutes a "major" human metabolite. In the absence of such consensus, it is unclear how to apply the results of measurements in various biological matrices to the design of subsequent toxicity studies and the establishment of safety margins for human use.

Extent of metabolite safety testing that is required

If testing of drug metabolites is required, general toxicity tests ranging from 14-90 days should be sufficient, along with genetic toxicity testing and a safety pharmacology evaluation to assess the potential for QT prolongation, as appropriate. Longer term testing will require large amounts of the synthesized metabolite and will unduly delay drug development without a commensurate increase in the value of the safety assessment program. There is published literature that demonstrates that additional findings of toxicological significance in study durations beyond 90 days are of infrequent value[10].

Lack of clarity in the nonclinical testing required to provide adequate metabolite coverage

It is not clear from the draft Guidance whether adequate systemic exposure to each human metabolite is required in both rodents and nonrodents. Given the variability in the quantitative production of metabolites between species, we suggest that an adequate margin of metabolite exposure in one species should be sufficient, unless there is a dramatic difference in toxicity between species that is viewed as being related to metabolite exposure differences.

Metabolite coverage in genotoxicity testing

Rather than conducting a separate genotoxicity testing program with synthesized metabolites, adequate coverage in the *in vitro* genotoxicity assays may be produced by the use of induced rodent S9 activation systems. We suggest that this should be sufficient.

Structural alerts

Experience leads industry to believe that *in silico* predictors of genotoxicity may have some value in highlighting cases for increased vigilance. However, QSAR structural alerts should be viewed as one element on which to base a decision on the need for carcinogenicity testing only if confirmed by the results of a positive experimental test, since the *in silico* tools in common use produce a significant number of false positives. Regardless of whether QSAR calculations are performed, if actual testing is done, the experimental results should supplant *in silico* predictions in all cases.

---

[10] H. Olson, G. Betton, D. Robinson, K. Thomas, A. Munro, G. Kolaja, P. Lilly, J. Sanders, G. Sipes, W. Bracken, M. Dorato, K. Van Deun, P. Smith, B. Berger and A. Heller, Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulat. Toxicol. Pharmacol., **32**, 56-67 (2000).

Recommendation

In light of the potential profound effect the recommendations contained in the draft Guidance will have on drug development, we suggest that further dialog on the topic of safety testing of drug metabolites take place among experts and the Agency. Based on the comments provided herein and elsewhere, we recommend a second draft of this guidance be prepared, including a discussion of the scientific considerations involved with interpreting such nonclinical data. As written, the Guidance will have a negative impact on drug development without adding significant value.

We appreciate the opportunity to share our comments with respect to the FDA Draft Guidance for Industry on Safety Testing of Drug Metabolites. Please do not hesitate to contact me, should you have any questions.

Sincerely,

Taryn Rogalski-Salter, PhD
Director
Regulatory Policy